Earliest direct evidence of plant processing in prehistoric Saharan pottery

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The invention of thermally resistant ceramic cooking vessels around 15,000 years ago was a major advance in human diet and nutrition[1-3], opening up new food groups and preparation techniques. Previous investigations of lipid biomarkers contained in food residues have routinely demonstrated the importance of prehistoric cooking pots for the processing of animal products across the world[4]. Remarkably, however, direct evidence for plant processing in prehistoric pottery has not been forthcoming, despite the potential to cook otherwise unpalatable or even toxic plants[2,5]. In north Africa, archaeobotanical evidence of charred and desiccated plant organs denotes Early Holocene hunter gatherers routinely exploited a wide range of plant resources[6]. Here, we reveal the earliest direct evidence for plant processing in pottery globally, from the sites of Takarkori and Uan Afuda in the Libyan Sahara, dated to 8200-6400 calBC. Characteristic carbon number distributions and $\delta^{13}C$ values for plant wax-derived *n*-alkanes and alkanoic acids indicate sustained and systematic processing of C_3/C_4 grasses

and aquatic plants, gathered from the savannahs and lakes in the Early to Middle Holocene green Sahara.

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Diet is a driving force in human evolution, linked with the development of physiology together with ecological, social, and cultural change within the hominin lineage[1-3]. The processing of foodstuffs was a major innovation, with the cooking of plants a crucial step as this would have increased the availability of starch as an energy source and rendered otherwise toxic and/or inedible plants palatable and digestible[2,5]. The need for increased processing likely arose with the expansion in dietary plant diversity suggested by the increased complexity of plant palaeobotanical assemblages recovered from Pleistocene and Early Holocene hunter-gatherer sites across the world[7]. Specialisation in particular plants, notably cereals and pulses, is regarded as one of the characteristics of the Neolithic domestic agricultural "package" in the Near East and Europe, although the sequence and nature of plant and animal domestication varied markedly geographically.

This is particularly manifest in North Africa where the early Holocene green Sahara[8] comprised a mosaic of humid savannah with extensive herds of large fauna, interspersed with networks of rivers and lakes supporting aquatic plants and animals. The richness of the environment provided significant food procurement opportunities, initially for the semi-sedentary pottery-using hunter-gatherers of the region and then for the first pastoralists who exploited domesticated livestock, such as cattle, sheep and goat[9].

North Africa is one of the two known centres worldwide for the invention of pottery (*c.* 10,000 calBC), with East Asia (*c.* 14,000 calBC) being the other[10,11]. Crucially, pottery from two well-dated Libyan Saharan archaeological sites allows the investigation of plant processing as a dietary strategy over this period. Uan Afuda cave[12] was occupied by hunter-

gatherers during the period dated 8200-6700 BC and the Takarkori rock shelter is one of the few Saharan sites which records the transition from hunter-gathering (8200-6400 BC) to food production (6400-3000 BC), with nearly 5000 years of human occupation[13] (Supplementary information Figs. 1, 2 & 3; Map of Tadrart Acacus Mountains, Libya; Uan Afuda cave and Takarkori rock shelter). Both sites yielded sedimentary deposits extraordinarily rich in pollen and plant macrofossils, suggesting exploitation for human consumption[14,15]. At Takarkori, these included exceptionally well-preserved organs from plants such as *Typha, Ficus, Cupressus, Tragus, Cassia* and *Balanites aegyptica* (Fig. 1) together with Panicoideae fruits (e.g. *Echinochloa*, *Panicum* and *Setaria*). Significantly, pottery was also introduced around this time[10,11] presenting the unique possibility to explore plant exploitation and processing amongst these Holocene hunter-gatherer people through organic residues preserved in some of the regions earliest cooking vessels.

A total of 110 potsherds from Early to Middle Holocene contexts at Takarkori and Uan Afuda (Supplementary information Figs. 4 & 5) were solvent extracted using established protocols and analysed using GC, GC-MS and GC-C-IRMS[4,9]. Of the 81 sherds analysed from Takarkori, n = 29 displayed distributions typical of an animal fat origin[9], n = 38 displayed distributions strongly indicative of a plant origin (Late Acacus, n = 4; Early Pastoral, n = 2 and Middle Pastoral, n = 32; Supplementary Tables 1 and 2) with the remainder likely reflecting either the processing of both plant and animal products in the vessels or the multi-use of vessels. Potsherd samples from the Uan Afuda cave, Libya, all from Late Acacus stratigraphic contexts dated by multiple radiocarbon measures, totalled n = 29, of which n = 22 yielded appreciable lipid concentrations (76%). Of these, n = 18 of the TLEs yielded lipid profiles indicative of a plant origin (82%).

The lipid profiles from both sites are characterised by unusually complex mixtures of aliphatic compounds including short, medium and long-chain fatty acids, diacids, α,ω-

hydroxyacids and *n*-alkanes (Fig. 2). The exceptional preservation of lipids in the desert environment presented opportunities to use a range of diagnostic criteria and proxies to explore the nature of the lipid distributions in the pottery: palmitic/stearic acid ratios (P/S ratio), average chain length[16] (ACL), carbon preference index[17] (CPI), P_{aq} proxy ratio[18] and compound-specific $\delta^{13}C$ values are summarised in Table 1 (see also Supplementary Information Tables 1 and 2).

The saturated fatty acids seen in all gas chromatograms (Fig. 2a-c) are common degradation products of acyl lipids. Fresh fatty acids of plants are dominated by unsaturated components (such as $C_{18:1}$ and $C_{18:2}$) but these are either absent or greatly reduced in abundance in aged fats and oils due to oxidation. Well-known plant degradation products are evident in the gas chromatograms as short-chain fatty acids, such as *n*-nonanoic acid and diacids, e.g. azelaic acid. Strong evidence for plant lipids dominating the extracts comes from the high abundance of palmitic versus stearic acid expressed by high P/S ratios (>4), a pattern never seen in animal fats, especially those of archaeological origin[19]. The high abundance of lauric ($C_{12:0}$) and myristic ($C_{14:0}$) acids is very unusual as these compounds exist only at very low abundance in most plant lipids (Fig. 2c). They occur in high abundance in palm kernel oil[20-21] but the date palm was not thought to have been present in the Sahara at that time, its natural range in prehistory being restricted to Southwest Asia. Seed oil chain lengths can range from 8 to 24 carbons, with degrees of unsaturation ranging from 0 to 4[20-22]. Likely candidates for seed oil processing in the vessels might be both C_3 and C_4 wild grasses, ubiquitous in the archaeological deposits at both sites. The high P/S ratios of these residues also suggest that oil was processed in the pots[23], and interestingly, some vessels with high P/S ratios do not include *n*-alkanes, denoting the presence of plant waxes, suggesting the dedicated processing of plant fruits and seeds rather than leafy plants or stems.

However, the presence of long-chain fatty acids up to C_{30} is strongly indicative of an origin in leaf or stem epicuticular waxes, although such compounds are also found in suberin[24], an aliphatic polyester found in all plants. Overall, the different distributions of fatty acids points to extensive processing of a range of different plant types and organs, such as grains/seeds and leafy plants and stems, in the pottery.

The abundant *n*-alkanes also derive from plant epicuticular waxes, with two main signatures dominating the extracts: i.e. either medium chain-length *n*-alkanes, C_{25} or C_{27}, or longer chain *n*-alkanes, namely the C_{31} *n*-alkane (Fig. 2a,b). Comparison to the archaeobotanical record from the sites, and known affiliations, suggests the lipid profiles dominated by C_{31} *n*-alkanes likely originate from C_3 or C_4 wild grasses or lake-margin plants, such as sedges[25-27]. However, lipid profiles with typical *n*-alkane distributions maximising at C_{25} are highly unusual (Fig. 2a) and more diagnostic to plant type. A predominance of C_{23} and C_{25} *n*-alkanes is known to be characteristic of submerged and floating aquatic plants[18,27], such as *Potamogeton*[28], also found in the pollen records in the region[29]. Calculation of the previously proposed P_{aq} proxy ratio further confirmed the lipid profiles with C_{25} *n*-alkane maxima likely originating from aquatic plants (Table 1 and Supplementary information Table 1), with P_{aq} ratio values between 0.4-1.0 indicative of submerged or floating macrophytes at both sites. It is especially significant that continuity is evident in the processing of aquatic plants in pottery spanning the Early to Middle Holocene, which includes the transition from hunter-gathering to pastoralism.

The extremely broad range of $\delta^{13}C$ values for both the alkanoic acids and *n*-alkanes confirms mixtures of C_3 and C_4 plants were being processed in the vessels (Fig. 3a,b and Supplementary Information Table 1). The individual $\delta^{13}C$ values for the leaf wax *n*-alkanes from both sites range from -30.0 to -17.7 ‰ for the C_{25} *n*-alkane, from -32.6 to -23.1 ‰ for the C_{31} *n*-alkane and from -27.4 to -13.8 ‰ for the $C_{16:0}$ fatty acid. These ranges reflect the

known $\delta^{13}C$ values for both bulk plant lipids (from -32 to -20 ‰ for C_3 plants and from -17 to -9 ‰ for C_4 plants[30]) and for leaf wax lipids, which are more depleted in ^{13}C than the biomass (between -39 and -29 ‰ in C_3 plants and -26 and -14 ‰ in C_4 plants[31]). These ranges also encompass the carbon isotope values of freshwater aquatic plants, which commonly display a C_4-like signature[32] but, as discussed above, are separable based on their respective *n*-alkane distributions.

Hence, the biomarker and stable isotope evidence from the pottery are entirely consistent with the archaeobotanical record, which comprises plants commonly found in the savannah and freshwater habitats present in the Holocene green Sahara (Supplementary Information Fig. 6). What is especially significant is that this is the first evidence that these plants were being processed in pottery vessels at least 10,000 years ago, with a prevalence of plant over animal lipid residues (54% of the total residues recovered from the vessels have a predominantly plant source, with the remainder comprising animal fats or mixtures of plant and animal products) in the pottery assemblages, emphasising the importance of a wide variety of plants, including grains/seeds, leafy and aquatic plants, in these prehistoric people's diet. Significantly, although the archaeobotanical record across North African sites suggests the consumption of plantstuffs such as cereals (seeds) and sedges, confirmed by these data, the role of aquatic plants in the diets of these prehistoric groups was not previously known. This exploitation of such a variety of plants highlights the sophistication of these early hunter-gatherer groups. Specific examples of where the pottery lipid and archaeobotanical records converge include: (i) evidence for different parts of *Typha* or cattail, found at Takarkori (Fig. 1a) and Uan Afuda, including rhizomes, peeled stems, flower spikes and pollen, which are known to have been exploited as a food source across the world[6,33], and (ii) consumption of leaves, stems and starchy edible rhizomes of some *Potamogeton*[34]. Processing of this type of emergent flora has a long history of use in north Africa[35], based on

finds of carbonised rhizomes of several sedges (*Cyperus rotundus*, *Scirpus maritimus* and *S.tuberosus*) at Wadi Kubbaniya, Egypt, *c*. 17,000 to 15,000 BC. Grindstones, ubiquitous in north African archaeological deposits, and abundant in the archaeological layers at Uan Afuda and Takarkori, would have facilitated the processing of these wild plants.

In summary, these findings provide unequivocal evidence for extensive early processing of plant products in pottery vessels, likely invented in this region for this purpose[10,36]. The higher frequency of plant product processing compared to animal products is unique in prehistoric pottery assemblages. From a temporal perspective the results indicate prolonged processing of a broad range of plant material within vessels, dating from the Early Holocene. This is contemporaneous with the introduction of pottery in the region and continues for over 4,000 years. Viewed together, this highlights the sophistication of both food procurement strategies and processing techniques of early Holocene north African foragers, having important implications for dietary security in the changing environments of the green Sahara. Ultimately, the adoption of these broad resource economies, together with a 'package' of ceramic containers, stone tools, grinding equipment, and storage facilities, were the cultural prerequisites for the rapid adoption of domesticated animals in North Africa. Interestingly, these data demonstrate that plant processing maintains its importance in the subsistence strategies of these prehistoric groups, occurring both contemporaneously with, and following, the adoption of domesticates and the exploitation of secondary products[9].

Significantly, African plant domestication did not occur until much later, around 2500BC, likely in part because the mid-Holocene savannah provided sufficient wild-growing grains and other plants to meet the people's dietary needs. Finally, adoption of these new plant processing techniques, using thermally resistant ceramic cooking vessels, would also have had far-reaching implications for improvements in human nutrition, health and energy gain. Critically, significant evolutionary advantages would have accrued through the provision of

cooked foods, soft enough to be palatable for infants, potentially leading to earlier weaning and shorter interbirth intervals, thereby enhancing the fertility of women in early pastoral communities.

1944 words

Method section

Lipid analysis and interpretations were performed using established protocols described in detail in earlier publications[4,9]. All solvents used were HPLC grade (Rathburn) and the reagents were analytical grade (typically > 98% of purity). Briefly, ~2 g of potsherd were sampled and surfaces cleaned with a modelling drill to remove any exogenous lipids. The sherds were then ground to a powder, an internal standard added to enable quantification of the lipid extract (*n*-tetratriacontane, typically 40 μg) and solvent extracted by ultrasonication (chloroform/methanol, 2:1 *v/v*, 2 x 10 ml). The solvent was evaporated under a gentle stream of nitrogen to obtain the total lipid extract (TLE). Aliquots of the TLE were trimethylsilylated (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide, Sigma Aldrich, 80 μl, 70 °C, 1 hr) and then analysed by high-temperature gas chromatography (HTGC) and gas chromatography-mass spectrometry (GC/MS) to identify the major compounds present. All TLEs were initially screened in a Agilent Industries 7890A GC system equipped with a fused-silica capillary column (15 m × 0.32 mm) coated with dimethyl polysiloxane stationary phase (DB-1HT; film thickness, 0.1 μm; Agilent Technologies). Derivatized extracts (1.0 μl) were injected on-column using a cool on-column inlet in track oven mode. The temperature was held isothermally for 2 min at 50 °C and then increased at a rate of 10 °C min^{-1} and held at 350 °C for 5 minutes. The flame ionization detector (FID) was set at a temperature of 350 °C.

Helium was used as a carrier gas, set to a constant flow (4.6 ml min^{-1}). Data acquisition and processing were carried out using the HP Chemstation software (Rev. B.03.02 (341), Agilent Technologies).

GC/MS analyses of trimethylsilylated aliquots were performed using a ThermoFinnigan TraceMS operating at 70 eV with a scanning range of 60-600 Daltons. Samples were introduced by on-column injection. The analytical column (15 m x 0.32 mm) was coated with dimethyl polysiloxane (ZB-1; film thickness, 0.12 µm). The temperature programming was from 50 to 300 °C at 10°C min^{-1}, following a 2 min isothermal hold at 50 °C. At the end of the temperature programming the GC oven was kept at 300 °C for 10 minutes. Helium was used as the carrier gas. Data acquisition and processing were carried out using XCalibur software (version 2.0.6). Peaks were identified on the basis of their mass spectra and gas chromatography (GC) retention times, by comparison with the NIST mass spectral library (version 2.0).

Further aliquots of the TLE were treated with $NaOH/H_2O$ (9:1 *w/v*) in methanol (5% *v/v*, 70 °C, 1 h). Following neutralization, lipids were extracted into chloroform and the excess solvent evaporated under a gentle stream of nitrogen. Fatty acid methyl esters (FAMEs) were prepared by reaction with BF_3-methanol (14% *w/v*, Sigma Aldrich, 70 °C, 1 hr). The FAMEs were extracted with chloroform and the solvent removed under nitrogen. The FAMEs were re-dissolved into hexane for analysis by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS).

The majority of carbon isotope analyses were carried out by GC-C-IRMS using an Agilent 6890 GC gas chromatograph, with a CTC A200S autosampler, coupled to a Finnegan MAT $Delta^{plus}$ XL mass spectrometer *via* a Finnigan MAT GCCIII interface. Samples were injected *via* a PTV injector in splitless mode, with a temperature increasing from 70 °C to 300 °C. The

GC was fitted with a Varian fused silica capillary column (CP-Sil5CB, 100% dimethylpolysiloxane with 0.12 µm film thickness, 50 m x 0.32 i.d.). Helium was used as the carrier gas at a flow rate set at 2 mL min^{-1}. Cu, Ni, Pt (0.1 mm) were used in the alumina combustion reactor (0.5 mm i.d.). The combustion reactor temperature was maintained at 950 °C. The temperature programme comprised of a 2 min isothermal period at 50 °C increasing to 250 °C at a rate of 10 °C min^{-1}, followed by an isothermal period of 15 min at 250 °C. Faraday cups were used to select ions of *m/z* 44 ($^{12}C^{16}O_2$), *m/z* 45 ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$) and *m/z* 46 ($^{12}C^{18}O^{16}O$).

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End notes

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Table and figures captions

Table 1. P/S ratios, CPI, ACL, weighted mean, P_{aq} values and classifications of trimethylsilylated total lipid extracts from Takarkori rock shelter and Uan Afuda cave.

Sample no.	Archaeological phase	P/S ratio	CPI C_{23}-C_{33}	ACL C_{23}-C_{33}	Weighted mean $\delta^{13}C$	P_{aq}	Classification
TAK4	Late Acacus	3.1	2.1	27.7	-23.2	0.55	Aquatic plant
TAK14	Middle Pastoral	2.0	n/d	28.9	-29.2	n/d	C_3 grass
TAK23	Late Acacus	2.6	5.7	28.7	-21.4	n/d	C_4 grass or aquatic
TAK24	Early Pastoral	5.4	n/d	28.5	-22.4	n/d	C_4 grass or aquatic
TAK82	Middle Pastoral	2.8	8.1	28.5	-21.2	n/d	C_4 grass or aquatic
TAK135	Middle Pastoral	5.0	n/d	28.2	-23.3	0.55	Aquatic plant
TAK159	Middle Pastoral	4.2	n/d	27.4	-20.7	0.61	Aquatic plant
TAK479	Middle Pastoral	4.6	5.2	27.7	-27.2	0.58	Aquatic plant
TAK709	Middle Pastoral	2.5	3.9	29.4	n/d	n/d	C_4 grass
TAK730	Middle Pastoral	4.8	5.1	28.9	-25.0	n/d	C_3 grass
TAK766	Middle Pastoral	2.9	8.1	26.3	-19.5	0.80	Aquatic plant
TAK860	Middle Pastoral	4.0	n/d	28.6	-21.4	n/d	C_4 grass or aquatic
TAK873	Middle Pastoral	2.0	4.6	29.5	-22.4	n/d	C_4 grass/sedge
TAK953	Middle Pastoral	2.1	7.6	28.9	-24.0	0.35	C_4 grass/sedge
TAK1008	Middle Pastoral	2.5	n/d	27.9	-26.3	n/d	Aquatic plant?
TAK1054	Middle Pastoral	3.1	n/d	28.4	-23.9	0.58	Aquatic plant
TAK1072	Middle Pastoral	2.9	n/d	28.1	-20.7	0.57	Aquatic plant
TAK1531	Middle Pastoral	4.0	n/d	28.4	-24.9	n/d	Aquatic plant?
UAF A1	Late Acacus	3.7	n/d	29.2	-24.9	n/d	C_3 plant
UAFA3	Late Acacus	3.7	n/d	27.3	n/d	0.89	Aquatic plant
UAF20	Late Acacus	1.5	n/d	28.7	-21.5	n/d	C_3 plant
UAF45	Late Acacus	4.7	n/d	26.0	n/d	0.75	Aquatic plant
UAF46	Late Acacus	4.7	n/d	27.7	-26.4	n/d	Aquatic plant?
UAF50	Late Acacus	4.2	n/d	26.3	n/d	n/d	Aquatic plant?
UAF84	Late Acacus	14.3	n/d	26.9	n/d	n/d	Aquatic plant?

n/d - not determined, signal intensity too low
Late Acacus period 8900-7400 years uncalibrated years BP, 8300-6100 calBC
Early Pastoral 7400-6400 years uncalibrated years BP, 6300-5300 calBC
Middle Pastoral 6100-5000 years uncalibrated years BP, 5200-3900 calBC[12,13]
P/S ratio - relative abundance ratio $C_{16:0}/C_{18:0}$ fatty acids, values greater than 4 indicate a plant origin
CPI - measures the relative abundance of odd over even carbon chain lengths, e.g. CPI values for all plant species have strong odd-chain preferences, with CPI values varying between 1.6 and 82.1[17]
ACL - weight-averaged number of carbon atoms of the higher plant C_{25} - C_{33} *n*-alkanes[16]
P_{aq} - emergent and non-emergent aquatic macrophyte input, P_{aq}<0.1 corresponds to a terrestrial plant input, P_{aq} 0.1-0.4 to emergent macrophytes and P_{aq} 0.4-1.0 to submerged or floating macrophytes[18]

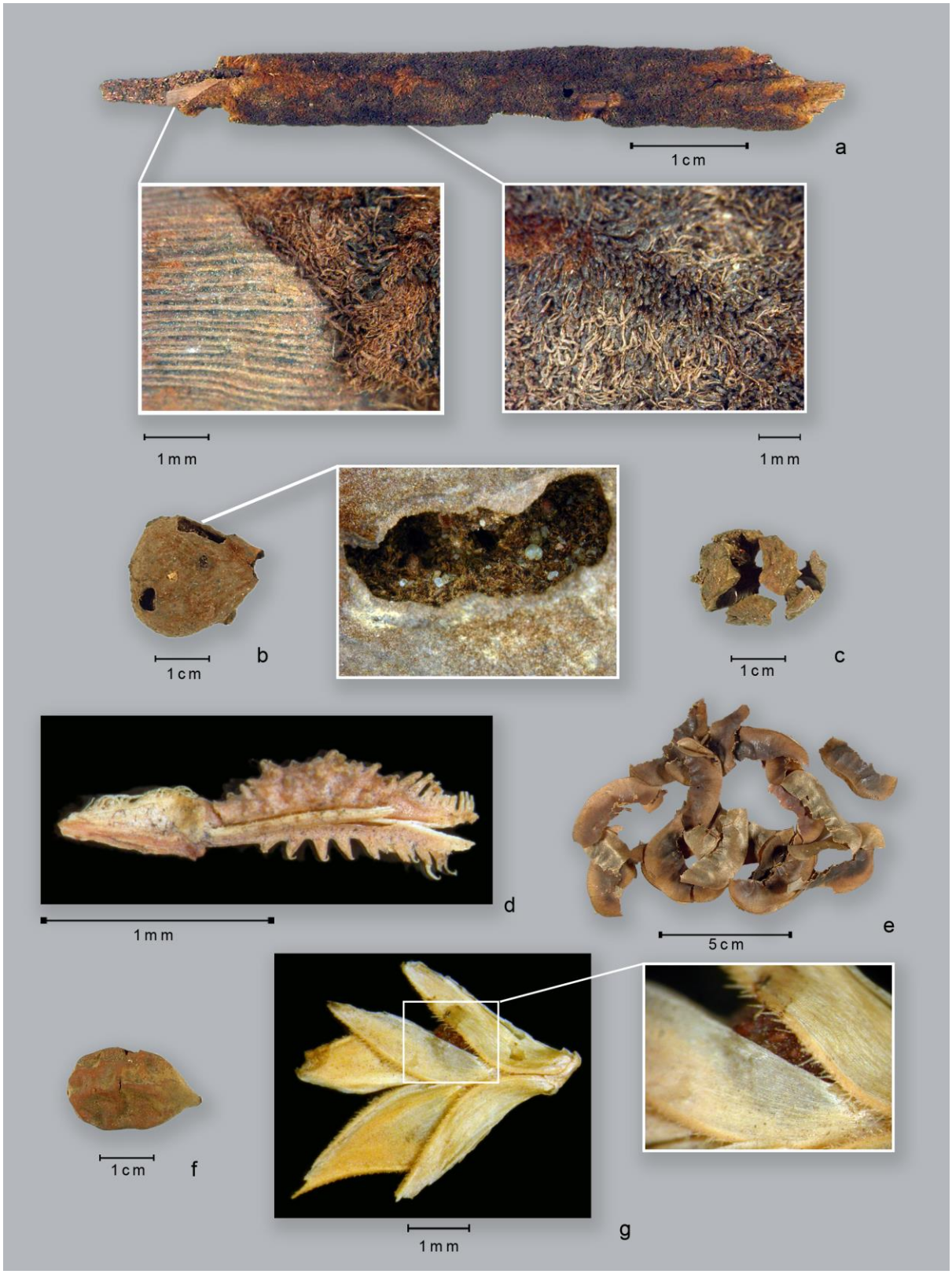

Figure 1 Exceptionally preserved archaeobotanical remains from Takarkori rock shelter (Tadrart Acacus, SW Libya), dating approximately from *c*. 7500 to 4200 calBC (a) Inflorescence of *Typha* (Late Acacus 3 - *c*. 6800 calBC), (b) Syconium of *Ficus* sp., and

details (Late Acacus 2 - *c*. 7500 calBC), (c) Galbulus of *Cupressus* (Middle Pastoral 2), (d) spikelet of *Tragus* (Middle Pastoral 2 - *c*. 4200 calBC), (e) legumes of *Cassia* (Early Pastoral 1 - *c*. 6350 calBC), (f) fruit of *Balanites aegyptica* (Late Acacus 3 - *c*. 6800 calBC), (g) spikelet of *Dactyloctenium aegyptium* and details of grain (Middle Pastoral 2 - *c*. 4200 calBC). (© The Archaeological Mission in the Sahara, Sapienza University of Rome)

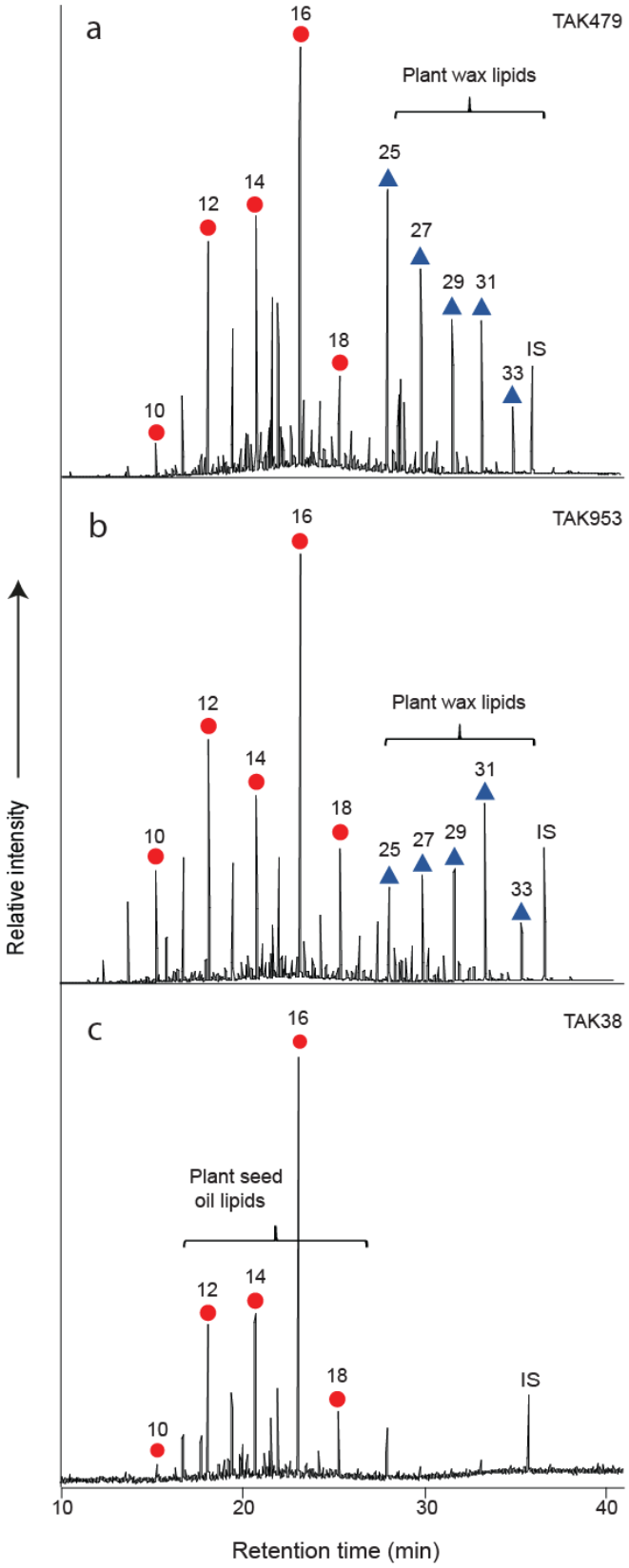
a
TAK479
16
Plant wax lipids
25
14
12
27
29
31
18
IS
33
10
b
TAK953
16
12
Plant wax lipids
14
31
18
10
25
27
29
IS
33
c
TAK38
16
Plant seed
oil lipids
12
14
18
IS
10
Relative intensity
10
20
30
40
Retention time (min)

Figure 2 Partial gas chromatograms of trimethylsilylated total lipid extracts (TLEs) from potsherds excavated from Takarkori rock shelter. a-c, Chromatographic peak identities denoted by filled triangles comprise *n*-alkanes in the carbon change range $C_{25:0}$ to $C_{33:0}$ and filled circles indicate straight-chain fatty acids in the carbon chain range $C_{9:0}$ to $C_{30:0}$, maximising at $C_{16:0}$. a-c, the distributions show leaf wax *n*-alkanes and plant fatty acids (a) *n*-alkanes maximising at C_{25} characteristic of an aquatic plant origin, (b) *n*-alkanes maximising at C_{31} originate from C_3 or C_4 wild grasses or lake-margin plants, such as sedges, and (c) plant fatty acid profile showing the predominance of the $C_{16:0}$ over the $C_{18:0}$ fatty acid and high abundance of $C_{12:0}$ and $C_{14:0}$ fatty acids, characteristic of plant seed oil lipids. IS, internal standard, C_{34} *n*-tetratriacontane.

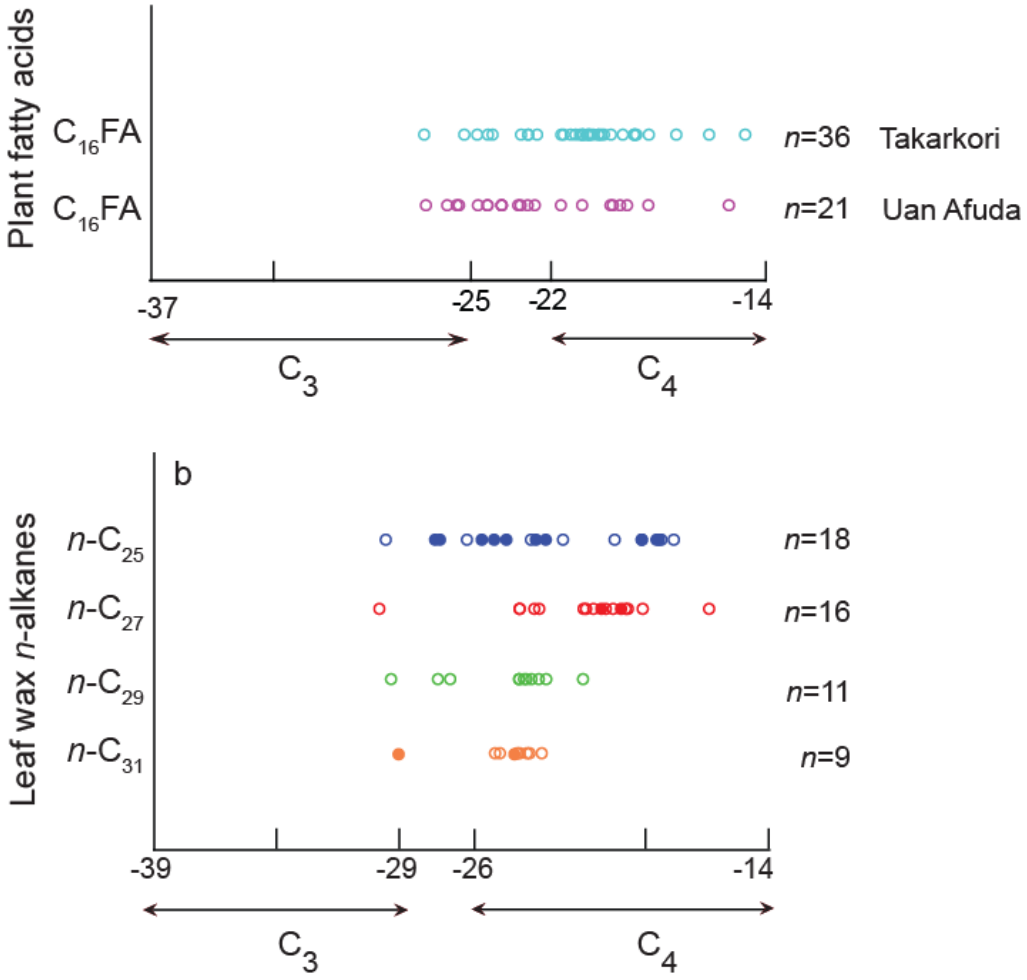

Figure 3 Plot showing range of $\delta^{13}C$ values for the alkanoic acids and *n*-alkane lipids derived from absorbed residues preserved in pottery from the Uan Afuda cave and Takarkori rockshelter, Libyan Sahara. These $\delta^{13}C$ values confirm a combination of C_3 and C_4 plants were being processed in the vessels. The ranges reflect the known $\delta^{13}C$ values for both bulk plant lipids (from -32 to -20 ‰ and from -17 to -9 ‰ for C_4 plants[30]) and for leaf wax lipids which are more depleted in ^{13}C than the biomass (between -39 and -29 ‰ in C_3 plants and -26 and -14 ‰ in C_4 plants[31])